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Chapter 7



Biological removal of sulfurous compounds and metals from inorganic wastewaters

David Barrie Johnson and Ana Laura Santos

7.1 INTRODUCTION

Sulfur-rich wastewaters that contain relatively little dissolved organic carbon may be generated as a result of several industrial processes: for example, galvanic processes, scrubbing of flue gases at power plants and detoxification of metal-contaminated soils (Table 7.1). However, the mining of heavy metals and coal is, by far and away, responsible for generating the bulk of this type of industrial wastewater (Blowes *et al.*, 2014). Sulfate- and metal-rich effluents produced at the sites of active and derelict mines are frequently referred to as ‘acid mine (or rock) drainage’ (AMD/ARD). ARD can also be found at sites that have not been impacted by mining, such as gossans in the high Arctic, and upstream of the large and historic mining works at the Rio Tinto, Spain. The pollution of global watercourses due to AMD is immense and occurs in countries that have legacies of historic as well as current mining operations. In the 1990s, it was estimated that there were between 20,000 and 50,000 mines releasing AMD in US Forest Service lands alone (USDA, 1993), impacting many thousands of kilometers of streams and rivers. Flooding of voids left by opencast mining creates ‘pit lakes’ that have variable chemistries (depending on the ore that was mined, and the local geology) but these are also often acidic and enriched with metals and sulfate (Geller *et al.*, 1998; Sánchez España *et al.*, 2013).

Table 7.1 Examples of inorganic sulfate-containing wastewaters produced by industrial activities.

Wastewater Origin	Sulfate (g/L)	Sulfite (g/L)	Other Pollutants	Reference
Industrial activity				
Chemical industry	0.2–50	0–5		Stucki <i>et al.</i> (1993)
Mining industry	0.1–50	–	Heavy metals	Banks <i>et al.</i> (1997)
Galvanic industry	0.2–50	0–25	Heavy metals	Tichy <i>et al.</i> (1998)
Flue-gas scrubbing	1–2	1–2		Dijkman (1995)

7.2 SULFUR-RICH WASTEWATERS ASSOCIATED WITH MINING ACTIVITIES

There is a long history of sulfur-rich wastewater production resulting from mining activities; for example, the Iberian pyrite belt has been associated with mining activities for over 5000 years (López-Archilla *et al.*, 1995). While not all mining activities result in AMD production, operations targeting base and precious metals and coals (lignite and anthracite) have been widely implicated for generating most of this form of environmental pollution. Many AMD streams have low pH, and contain elevated concentrations of sulfate, dissolved transition and other metals and occasionally metalloids (such as arsenic). The chemical compositions of these wastewaters can vary greatly on a regional basis and from site to site within a region (Table 7.2).

7.2.1 Origin of acid mine drainage

The major cause of acid mine drainage pollution is the (microbially) accelerated oxidative dissolution of sulfide minerals and the transportation of the products of these and of secondary reactions in flowing water. Coal contains both inorganic sulfur (principally iron sulfides and sulfate) and organic sulfur (chiefly thiol, thiophene, sulfoxide and sulfone), the relative proportions of which vary with different ranks of coal and degrees of oxidation. The sulfur content of coals is generally between 1 and 10% (Dugan *et al.*, 1991). Many metals of commercial importance, such as copper, cobalt, zinc and lead occur chiefly in the lithosphere as, or are associated with, sulfide minerals. Although the most abundant of all sulfide minerals, pyrite (FeS_2), is no longer mined as a major source of elemental sulfur, it (and other iron sulfide minerals) is a major gangue mineral in many metal ore bodies. Significant amounts of iron sulfides can be present in waste rock dumps and, in particular, in mine tailings produced by froth flotation, where the milling of ores further compounds the reactivity of the waste by reducing the size of particles and increasing their surface areas.

Table 7.2 Examples of mine water chemistries draining from metal and coal mines.

	Parys Cu Mine Wales (Abandoned)	Sossego Cu Mine Brazil (Active)	Curilo Uranium Bulgaria (Abandoned)	Mauritiden Zn Mine Sweden (Active)	Minsterley Brook Pb/Zn Mine England (Abandoned)	Bullhouse Coal Mine England (Abandoned)
pH	2.5	5.0	2.15–4.15	2.3	7.1	5.9
SO ₄ ²⁻ (mg/L)	3100	1505	341–1784		768	
Fe _{total} (mg/l)	650	0.15	73–1072	403	<0.01	61
Al (mg/L)	70	0.98		132		1.2
Mn (mg/L)	10	8.74	0.95–55	49		15
As (mg/L)				1.3		
Cu (mg/L)	60	477–483	0.41–14.9	7.7	<0.01	<1
U (mg/L)			0.28–4.82			
Zn (mg/L)	40	1.02	0.80–24.2	464	58	<1
Reference	Johnson (2006)	Santos and Johnson (2017)	Groudev <i>et al.</i> (2008)	Hedrich and Johnson (2014)	Holanda and Johnson (2020)	Johnson (2006)

Sulfide minerals are stable in situations where both oxygen and water are absent or excluded, such as an undisturbed ore body or coal seam. However, in moist aerobic environments they are unstable, and can oxidize spontaneously to form (ultimately) sulfate, the free metal or metalloid (which may be subject to subsequent hydrolysis and precipitation) and, if the net metal:sulfur ratio is <1 and the pH is >1.9 , hydronium ions. Several factors determine the rate at which sulfide minerals oxidize, such as the type of mineral and its surface area. Some species of lithotrophic ('rock eating') prokaryotes can, in low pH aerated and moist environments, accelerate the oxidation of sulfide minerals by factors of 10^4 to 10^6 and therefore render the spontaneous chemical oxidation of these minerals of little importance under these conditions. While in the 1950s, only one bacterial species was thought to be able to do this, it is now known that a large number of biodiverse acidophilic bacteria and archaea, acting alone or even more efficiently in consortia, can accelerate the oxidative dissolution of pyrite and other sulfide minerals (Quatrini & Johnson, 2016). The ability of these microorganisms to release or expose base and precious metals from sulfidic ores has been harnessed since the 1960s in a technology referred to as 'biomining', which operates in a variety of engineering configurations (waste dumps, bioheaps and stirred tanks) in countries throughout the world (Rawlings & Johnson, 2007).

The mechanism(s) by which bacteria actually solubilize these hard, dense minerals has been the subject of much debate. While molecular oxygen can act as the primary oxidant of sulfides, ferric iron is thought to have a more significant role, especially at low pH (<3) where this form of iron becomes increasingly soluble (Evangeliou, 1995; Vera *et al.*, 2013). Ferric iron is reduced to ferrous when it reacts with sulfide minerals. In the case of pyrite, six ferric ions are required to oxidize one ' FeS_2 ', with the resultant partial oxidation of one of the sulfur atoms in the sulfide (S^{-1}) moiety to thiosulfate ($\text{S}_2\text{O}_3^{2-}$, where the sulfur atoms have oxidation states of $+5$ and -1 ; Steudel, 2000).

Since spontaneous chemical oxidation of ferrous iron proceeds very slowly at low pH, microbiological oxidation is of critical importance, and iron-oxidizing acidophiles are therefore the primary agents involved in the oxidative dissolution of sulfidic minerals at low pH. The thiosulfate generated is unstable in low pH liquors and transforms to elemental sulfur and a variety of sulfur oxy-anions, all of which can be oxidized by sulfur-oxidizing prokaryotes (Quatrini & Johnson, 2016), forming sulfuric acid, which helps to maintain the pH conducive for the iron-oxidizers. A third group of acidophiles also contributes indirectly to the process by catabolizing organic materials arising as exudates or lysate from the (mostly) carbon-fixing iron- and sulfur-oxidizers. A schematic outlining how consortia of these three groups of acidophiles interact during the oxidative dissolution of sulfide minerals is shown in Figure 7.1.

The complete chemical oxidation of pyrite that generates soluble ferric iron and sulfate (and/or bisulfate) can generate, or consume, proton (hydronium ion) acidity, depending on solution pH, since the pK_a of sulfate/bisulfate is 1.9

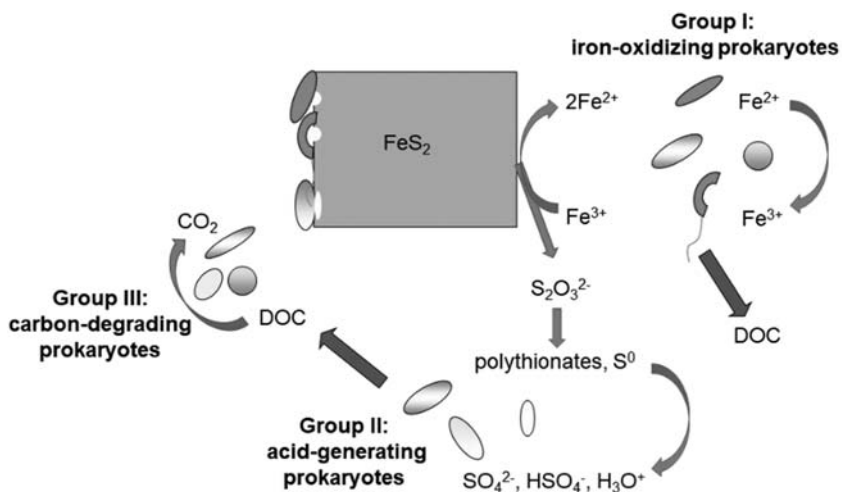
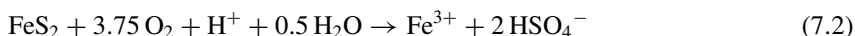
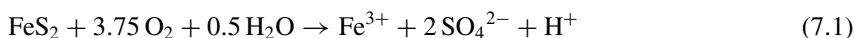
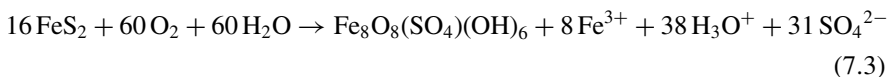


Figure 7.1 Interactive roles of acidophilic microbial consortia in the oxidative dissolution of pyrite. DOC, dissolved organic carbon.

(reactions (7.1) and (7.2)):



However, some or all of the ferric iron generated from pyrite oxidation can undergo mineralization reactions to form secondary oxidized minerals such as jarosites, ferrihydrite or (as shown in reaction (7.3)) schwertmannite:



This reaction is strongly acid-generating, which both limits the extent of secondary mineralization to form schwertmannite and provides a strong pH buffer to acidic iron-rich water bodies, helping to maintain their pH often within the range 2.2–2.8. This is most dramatically demonstrated in the Rio Tinto in south-west Spain, which remains within this pH range for most of its 60 km length ([Garcia-Moyano et al., 2012](#)).

Figure 7.2 illustrates the major point sources of AMD at a hypothetical abandoned deep mine where waste rocks have been deposited in heap dumps and mill wastes in tailings deposits. During the active life of mines, water tables are maintained at artificially low levels by pumping water to the surface. However, when mines are abandoned this is discontinued, and water levels rebound at

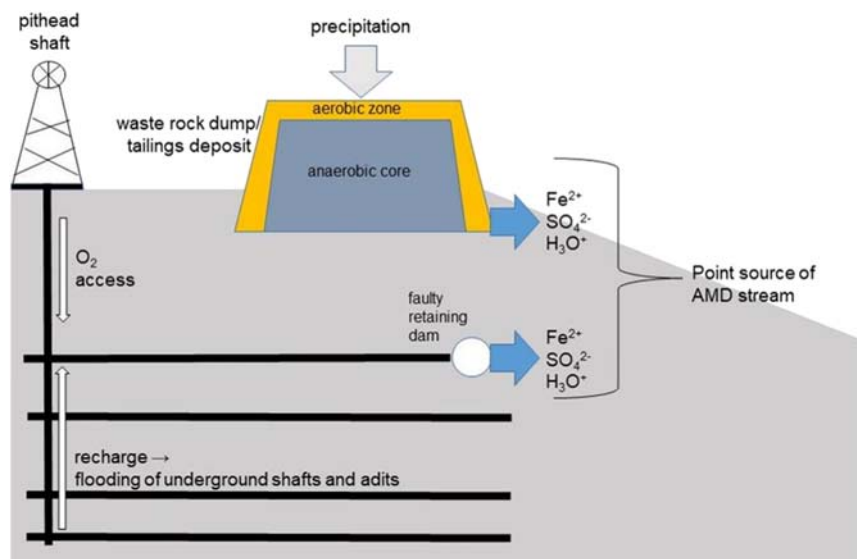
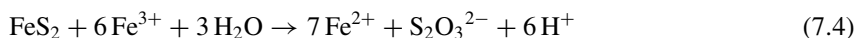


Figure 7.2 Sources of discharge of acid mine drainage at a hypothetical abandoned deep mine and associated mine waste deposits.

varying rates. The humid aerated atmosphere in the drained mine provides favorable conditions for sulfide mineral oxidation, and the up-welling waters cause iron, sulfate and other oxidation products to come into solution, so that the initial breakout of water tends to be the most acidic and polluting. Flooding mines limits oxygen diffusion rates and therefore sulfide oxidation, though this may be modified by fluctuating water tables. In the case of waste rock dumps and tailings deposits, aerated surface layers frequently overlie anaerobic deeper layers. In the aerobic region, sulfide mineral oxidation may proceed rapidly due to the microbially-accelerated reactions outlined above. However, the oxidation of sulfidic minerals can also occur within the anaerobic regions of waste dumps and tailings deposits due to the anoxic attack of soluble ferric iron present in waters flowing from the aerobic regions (7.4):



Waters emanating from these deposits are, therefore, often initially dominated by ferrous rather than ferric iron at the point of discharge. Aeration of these drainage waters facilitates the microbiological oxidation of the iron, giving rise to the characteristic deposition of orange-brown 'ochre' (or 'yellow boy') in affected streams and rivers (Figure 7.3).



Figure 7.3 Acidic, iron- and sulfate-rich water draining a waste rock dump at the abandoned San Telmo mine (Spain).

7.2.2 Chemical characteristics of AMD

Although the term ‘acid mine drainage’ is often used generically for iron- and sulfate-rich mine-impacted waters, their pH values may be close to neutral, particularly at the point of discharge. The total acidity of AMD comprises their measured hydronium ion concentrations (pH; ‘proton acidity’) and the hydronium ions that derive from the hydrolysis of dissolved metals, most notably iron, manganese and aluminum (‘mineral acidity’). As noted above, ferrous iron is often the dominant ionic form of this metal in oxygen-depleted waters emanating directly from underground mines or mine spoils and, unlike ferric iron, this species is soluble at circum-neutral pH. Oxidation of ferrous iron to ferric iron consumes one hydronium ion/iron oxidized, while the subsequent hydrolysis of iron (7.5) generates three hydronium ions/iron, so the net balance is two hydronium ions for the oxidation and subsequent precipitation of iron.



Similarly, the oxidation and hydrolysis of manganese is net productive of proton acidity, while in the case of aluminum (which exists only in the +3 oxidation state), net hydronium production is 3/aluminum hydrolyzed.

Mine waters that have pH values above 4.5 are considered to contain alkalinity due to the presence of (predominantly) dissolved bicarbonate. This can counter hydronium ion acidity, as shown in reaction (7.6):



The total acidity of discharged AMD can be calculated from measuring the concentrations of iron, manganese and aluminum. If this exceeds the bicarbonate alkalinity, the waters are assumed to be net acidic.

Besides acidity, other components of AMD are highly variable, as illustrated in Table 7.2. Elevated concentrations of iron and sulfate (compared to non-polluted surface waters) are their most characteristic feature, but other metals and metalloids may also be present at relatively high concentrations. The latter may also derive from the oxidation of sulfide minerals (e.g. copper from chalcopyrite; arsenic from arsenopyrite) or from accelerated weathering of non-sulfide minerals (e.g. aluminum from aluminosilicates). The solubility of most (cationic) metals, including some of the more toxic metals such as cadmium and lead, is far greater in acidic than in neutral and alkaline waters. The more acidic mine streams may therefore act as conduits for distributing metals to watercourses (including groundwater) considerable distances away from their sites of origin.

Redox potential measurements are a particularly useful physico-chemical index of AMD chemistry, since their E_h values are dictated by one of two major redox systems that occur in this type of polluted water: the ferrous/ferric couple and the sulfide/sulfate couple (Nordstrom *et al.*, 1979). Their E° values are affected by pH, both becoming more electronegative as pH increases. The E° value of the ferrous/ferric couple in acidic, sulfate-rich waters is about +710 mV (Johnson *et al.*, 2017), so that values above this potential indicate the predominance of ferric iron over ferrous, and values below this potential the opposite.

Concentrations of dissolved inorganic carbon (DIC) in low pH (<3.5) AMD waters tend to be very low (<0.5 mg/L) due to the dissociation of carbonic acid and the low solubility of CO₂ in acidic waters. In acidic mining lakes, DIC concentrations in the hypolimnion may greatly exceed those in the epilimnion. Concentrations of dissolved organic carbon (DOC) also tend to be very small (generally <10 mg/L) and are comparable with those in neutral/non-acidic oligotrophic waters. Some organic carbon may originate from the degradation of, for example, wooden pit props in underground mines. Primary production in mine waters themselves is mediated either by acid-tolerant micro-algae or by chemolithotrophic bacteria (Johnson & Aguilera, 2019).

The dominant form of nitrogen in AMD is usually ammonium, though significant concentrations of nitrate may occur where explosives are used (Blowes *et al.*, 2014). Levels of total nitrogen in AMD tend to be well in excess of total phosphorus, mostly due to the adsorption of the latter onto ferric iron precipitates (Blowes *et al.*, 2014). This macronutrient is therefore often considered to limit primary production in mine-impacted waters.

7.2.3 Impact of AMD on the biosphere

Acid mine drainage waters have a deleterious impact on most aquatic organisms, though these effects may be immediate or protracted. The major effects are due

to: (i) presence of toxic metals and metalloids; (ii) low pH values of the more severely acidic waters; (iii) precipitation of iron-rich ochre deposits on stream sediments, and (iv) osmotic stress. AMD impacted watercourses tend to be devoid of fish (due to mortality, and also because of loss of spawning gravel), have limited biodiversity in planktonic and benthic organisms, and display lower rates of primary production, compared to non-polluted surface waters.

Toxic metals and metalloids in AMD are more often encountered in waters draining metal mines than in those draining coal sites (Table 7.2). In aquatic ecosystems, metal distribution, speciation, and bioavailability in sediments and the water column can contribute to AMD toxicity. Many of these metals and metalloids can also be hazardous to human and animal health, if exposed to high concentrations, due to their ability to persist in the environment for an extended period and to accumulate in different levels of the food chain, which may result in acute or chronic diseases (Simate & Ndlovu, 2014). For example, exposure to high concentrations of chromium can disrupt vital metabolic functions and cause damage to the nervous system and some types of cancer (Singh *et al.*, 2011).

In extremely acidic mine waters, proton acidity can have a direct toxic effect (e.g. to fish, promoting formation of mucus in gills) or an indirect toxic effect, by enhancing the solubility of metals. The deposition of ochre from ferruginous mine drainage resulting from oxidation and hydrolysis of iron can result in long stretches of downstream waters being impacted. These iron-rich precipitates smother stream and river sediments, impeding oxygen diffusion and killing the majority of benthic organisms. This may have a dramatic effect on the food chain in impacted streams and rivers, with salmonid species being particularly sensitive to this type of pollution (Thoreau, 2002). In faster flowing waters, the ochre and other metal precipitates may remain in suspension rather than settle out. In that case, the turbidity of the water can have a major impact on light penetration, and thus negatively affect algal biomass and biodiversity (Robb, 1994).

Numbers and biodiversity of phytoplankton in AMD lakes follow the same general trends as described above, though exceptions to this pattern have been reported. Some macrophytes may establish in AMD waters, though mechanisms of metal tolerance and uptake are still not fully understood. Sheoran & Sheoran (2006) found that some species of macrophytes, such as *Typha angustata*, *Desmostachya bipinnata* and *Saccharum bengalense* could tolerate high concentrations of several metals with no growth impairment. Elsewhere, Nixdorf *et al.* (1998) showed that primary production by phytoplankton in acidic mine lakes in Germany was likely to be more limited by the availability of light, inorganic carbon and phosphorus, rather than by the low pH.

Most microorganisms are also severely damaged or killed upon exposure to AMD. Microbial communities that develop in acid polluted streams are very different from those that are found in local non-polluted streams (Mills & Mallory, 1987). However, it is now appreciated that the biodiversity of obligately acidophilic microorganisms active in highly acidic (pH <3) metal-rich waters,

such as AMD, is much greater than was previously recognized, and includes chemolithotrophic (iron- and/or sulfur-oxidizing) and heterotrophic prokaryotes (bacteria and archaea) as well as eukaryotes, such as fungi, yeasts, algae and protozoa (Johnson & Aguilera, 2019). These microorganisms living in extreme acidity sometimes form communities with others of their own species, but also different species where a variety of interactions may occur. Most types of interactions between the indigenous microbiota in non-polluted ecosystems have also been observed in extremely acidic environments.

7.3 PREVENTION, CONTAINMENT AND TREATMENT OF AMD

In recent years, environmental agencies throughout the world have been implementing more strict regulations regarding disposal and discharge levels of industrial effluents. Several mechanical, chemical and biological approaches have been used to preclude the formation of AMD or, where this is not feasible, to remediate AMD-impacted waters. ‘Source control’ measures are designed to prevent or minimize AMD production, while ‘migration control’ is aimed at restricting the movement of contaminated waters.

7.3.1 Non-biological prevention and remediation systems

Most source control approaches aim to minimize or even eliminate AMD production by isolating potentially acid forming materials from exposure to either oxygen or water. Underground mine backfilling is one of the management techniques used to prevent the contact between air and sulfide minerals, therefore limiting their oxidation. The backfills (e.g. soil, sand materials, cement, and paste backfill) are placed into mine shafts, which not only limits air infiltration, but may also provide sufficient alkaline to neutralize acidity, which in turn improves underground conditions (Villain *et al.*, 2013). However, it is appropriate if they can be securely sealed, though this is often a more risk-laden strategy and is not feasible where underground passages are intricately connected, or where detailed and accurate knowledge of mine workings is unavailable. Underwater storage of mine tailings, in which water acts as a diffusion barrier to oxygen, may be effective, particularly when the tailings are covered with a layer of sediment to further limit oxygen penetration and reduce surface perturbations (Blowes *et al.*, 2014). Land based waste heaps may be sealed with covers of clay, plastic liners or organic materials (e.g. wood wastes), both to reduce oxygen access and (in the case of plastic liners and clay skins) to limit water percolation (Olds *et al.*, 2012).

Not all abandoned mines and mine spoils will produce acidic effluents, since some may be virtually devoid of acid forming minerals, and others may contain sufficient quantities of neutralizing materials (e.g. carbonates). Blending of potentially acid generating with acid consuming materials has been used to

produce environmentally benign composites (e.g. Miller *et al.*, 2010). Other techniques involve the formation of highly insoluble iron phosphates on the surfaces of oxidizing pyritic minerals by phosphate amendment (Mauric & Lottermoser, 2011). Anionic surfactants have been demonstrated to inhibit sulfide mineral-oxidizing bacteria, though the effectiveness of controlling AMD production by applying these chemicals tends to be highly variable (Loos *et al.*, 1989).

Techniques developed to control the formation of AMD have mostly proven to be costly, impractical or inefficient, and therefore studies have been directed towards migration control. The most widespread method used to remediate AMD is to aerate and add alkaline chemicals to neutralize pH and remove metals, a process generally described as ‘active’ treatment. Different neutralizing agents may be used, such as limestone, soda ash (NaCO_3), ammonium and sodium hydroxide. The major objective is to accelerate the oxidation of ferrous iron (for which active aeration is also often necessary) and to precipitate iron and other metals as their hydroxides and/or carbonates. Some removal of sulfate (as gypsum) is achieved when calcium-containing neutralizing materials are used. Although active treatment can provide effective remediation of AMD, it has disadvantages of high infrastructure and operating costs, and problems with adequate disposal of the bulky sludge produced. The latter tends to be highly voluminous, typically containing only 2–4% (by weight) of solids and may contain highly toxic metals (and arsenic) that can be remobilized and released again into the environment (Veloso *et al.*, 2012).

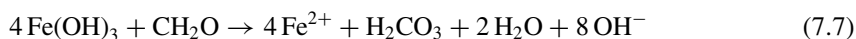
Alternative approaches for adding alkalinity to AMD include the use of anoxic limestone drains (ALDs), open limestone channels (OLCs), diversion wells and others (Skousen *et al.*, 2017). With ALDs, waters containing little or no dissolved oxygen (<1 mg/L) percolate through trenches or pits containing a layer of limestone and a plastic liner both underneath and also covering the limestone bed to avoid O_2 influx and CO_2 escape. Iron oxidation must be avoided with ALDs, as ferric iron deposits inactivate the limestone, a process known as ‘armoring’. Anoxic limestone drains require low maintenance, however metal removal must occur downstream of the drains or in association with biological treatment (e.g. constructed wetlands) especially in the cases of Al^{3+} and Fe^{3+} -rich AMD. Open limestone channels are streams lined with limestone rocks usually in areas with steep slopes into which contaminated water flows. Mine water is neutralized and oxidized by the OLC causing metal hydroxides precipitation. Precipitates may coat (or armor) the limestone surface, thus dissolution and acid neutralization may occur at lower rates than for uncoated limestone (Skousen *et al.*, 2017). These systems are more efficient when applied up-front in an integrated passive remediation system, where the AMD is most acidic.

Recent research has highlighted the potential use of ‘naturally available’ resources, such as crushed seashell, lignite, bentonite and attapulgite, for remediating AMD. Falayi and Ntuli (2014) reported the use of inactivated

attapulgite (a magnesium aluminum phyllosilicate) as an adsorbent for the removal of trace metals from a drainage of a gold mine. Over 95% of copper, nickel and ferrous iron were removed after a reaction time of 4 h.

7.3.2 Biological remediation systems

There are a number of biological processes that generate alkalinity (or consume acidity) and which therefore have potential use in remediating AMD. These include oxygenic photosynthesis, ammonification, and several reductive processes (methanogenesis, and iron and sulfate reduction). Of these, iron and sulfate reduction are obvious candidates for AMD bioremediation, given the chemical nature of AMD. Acid consumption resulting from the reduction of ferric iron depends on whether the latter is present in soluble or mineral form. Whilst the reduction of soluble ferric iron does not generate alkalinity, the reduction of amorphous and soluble ferric iron minerals does (7.7):



The reduction of sulfate to sulfide consumes protons in acidic solutions by transforming a strong acid (sulfuric) into a weak acid (hydrogen sulfide), as:



Both reactions require electron donors, which are generally organic ('CH₂O' in reactions (7.7) and (7.8)). An important additional feature of biological sulfate (as opposed to iron) reduction is that its product, sulfide, forms highly insoluble products with many toxic heavy metals that occur in AMD. The very low solubility products of many metal sulfides mean that these metals are removed highly effectively in the presence of only trace amounts of soluble sulfide (Diaz *et al.*, 1997). Dissimilatory reduction of sulfate can therefore fulfill three critical roles in remediating acidic sulfurous wastewaters: (i) increasing water pH, (ii) lowering concentrations of sulfate, and (iii) facilitating the (selective) precipitation of metals. In contrast, the dissimilatory reduction of elemental (or zero-valent) sulfur (ZVS) can mitigate only the last of these.

7.4 SULFATE REDUCTION IN MINE DRAINAGE WATERS AND OTHER EXTREMELY ACIDIC ENVIRONMENTS

The microbial ecology of metal-rich acidic environments, such as AMD, has been widely studied using both traditional microbiological and biomolecular approaches, sometimes in tandem. These have, however, mostly focused more on pure cultures and consortia responsible for accelerating the oxidative dissolution of sulfide minerals and thereby helping generate acidic, metal-rich wastewaters. Bacteria and archaea that are metabolically active in anaerobic low pH environments have received less attention, though it is known that many species

of acidophiles are able to grow via ferric iron respiration in oxygen-free or depleted zones (Coupland & Johnson, 2008). Fermentative and methanogenic prokaryotes are rare or unknown in extremely acidic environments, the former possibly because of the enhanced toxicity of their metabolic products (small molecular weight aliphatic acids) at low pH. Waters draining coal and metal mines often contain very elevated sulfate concentrations, and therefore it would be reasonable to expect that anaerobic sites (mostly sediments) in AMD streams and similar environments (e.g. mill tailings deposits, which are usually stored under water) would be populated with prokaryotes (mostly bacteria) that couple the oxidation of electron donors to the dissimilatory reduction of sulfate, generating hydrogen sulfide. However, it is interesting to note that up until the late 1990s, the only sulfate-reducing bacteria that had been isolated from acidic mineral sediments were neutrophilic strains that were highly sensitive to even moderate acidity. It was thought that these bacteria were inhabiting micro-sites that had much higher pH than implied by bulk measurements, and that they were active in maintaining the more (for them) conducive pH in these sites, as dissimilatory sulfate reduction is a proton-consuming reaction below \sim pH 6.

These early studies had been prompted by observations of hydrogen sulfide formation in mine-impacted water bodies. The first such report was by Tuttle *et al.* (1969) who described a situation where a mine water stream (pH 2.84) was constrained to flow through a porous dam containing wood dust into a receiving pond that was slightly less acidic (pH 3.38) and contained \sim 30% less sulfate. These chemical changes were ascribed to the activities of sulfate-reducing bacteria that were detected ($<10^3$ /mL) in the pond but not in the stream water, the implication being that these bacteria had been stimulated by organic compounds derived from the sawdust. Mixed bacterial populations reduced sulfate in the laboratory using sawdust as a carbon and energy source at pH 3.0, but none of the pure cultures of SRB they obtained were active below pH 5.5. Gyure *et al.* (1990) detected reduction of $^{35}\text{SO}_4^{2-}$ in sediment slurries in a pH 3.8 mine lake, though the optimum pH for sulfate reduction in sediment samples was found to be pH 5. Herlihy and Mills (1985) reported seasonal sulfate reduction occurring in freshwater sediments in lakes impacted by acid mine drainage (pH 2.5–3.5). There was no apparent point source of organic carbon, and dissolved carbon concentrations were not recorded, though water was described as 'eutrophic'. Fortin *et al.* (1996) and Fortin and Beveridge (1997) noted that sulfate reduction occurred in acidic (pH 3–4) mine tailings and isolated SRB from these environments, though again these were unable to grow in low pH (<5.5) media in the laboratory. Sulfate reduction was subsequently identified and quantified in extremely acidic (pH ≤ 3) sediments in a lake impacted by volcanism in Argentina by Koschorreck *et al.* (2003), acidic mine tailings in Canada (Praharaj & Fortin, 2004), Chile (Diaby *et al.*, 2007) and Siberia (Karnachuk *et al.*, 2005), moderately acidic (pH ~ 4) sediments around flooded mine workings in the USA (Church *et al.*, 2007) and sediments in the Rio Tinto, Spain (Sánchez-Andrea *et al.*, 2011).

Growths of ‘acid streamers’ have frequently been reported in flowing mine waters (Johnson & Aguilera, 2019). These are assemblages of bacteria and archaea, many of which oxidize ferrous iron, enmeshed in extracellular polymeric substances. Acid streamers are usually light cream/orange colored, though blackened growths may occasionally be found in sub-surface streamers. Johnson *et al.* (1993) added glycerol to cream-colored streamers and water, taken from an extremely acidic (pH 2.3–2.4) subterranean stream within an abandoned pyrite mine, and incubated them in the laboratory. An initial phase of iron reduction (marked by bleaching of the orange color of the mine water) was followed by a second phase of sulfate reduction (marked by the blackening of the cultures and the evolution of H_2S). This simple experiment inferred that acidophilic SRB were present in the streamer communities, but that their activities were restricted by the small amounts of organic compounds that could act as electron donors for sulfate reduction. This suggestion was supported by Rowe *et al.* (2007) who described a stream draining a small abandoned copper mine in the south of Spain that was being partially remediated by ‘natural attenuation’. The low pH and metal-rich drainage stream was rapidly colonized by a consortium of eukaryotic and prokaryotic acidophiles when it emerged from underground. These formed a thick multi-layered microbial mat, the upper layer of which was green colored due to the dominance of eukaryotic algae, while the lower zone was dark grey/black (due to accumulation of copper sulfide) and smelled strongly of hydrogen sulfide. Concentrations of dissolved organic carbon increased over three fold when the mine water passed over the surface algal growths, and were orders of magnitude greater in pore waters extracted from the lower (black) mat layers. Organic carbon derived from the surface algae streamers (as exudates and lysates) were thought to sustain the enhanced level of sulfate reduction in these macroscopic growths in comparison with the subterranean streamers where algae were absent.

7.4.1 Physiological constraints on sulfate- and sulfur-reduction

Sulfate is unique among the electron acceptors used by microorganisms in its necessity to be activated before it can be reduced (Hamilton, 1998). Sulfate activation consumes two adenosine triphosphate (ATP) molecules/sulfate, forming adenosine phosphosulfate which, in a two-electron reaction, is first reduced to sulfite, prior to a further six electron reduction to hydrogen sulfide (HS^- , and/or H_2S , depending on pH). In addition to this, transport of sulfate (in co-transport of H^+ or Na^+) into the cell is also energy-dependent. The redox potential of the sulfate/hydrogen sulfide couple ($E_h = -220$ mV, at pH 7) is much lower than many potential alternative electron acceptors, such as nitrate and ferric iron, though it is interesting to note that this E_h value actually becomes more electro-positive as the pH falls (by 74 mV/unit pH fall) and is +75 mV at

pH 3. Compared to aerobic microorganisms, and many anaerobes that use electron acceptors other than sulfate, SRB are severely disadvantaged with regard to the energy yield that is achievable from catabolism of organic substrates, which is further lowered in the case of SRB species that mediate incomplete oxidation of organic substrates and excrete small molecular mass organic metabolites, such as acetate. Standard redox potentials tend not to be identical to values experienced in actual metabolic situations as, under actual conditions, thermodynamic parameters might be modified by, for example, removal of reaction products in a linked series of reactions (i.e. syntrophism), resulting in dis-equilibrium (Hamilton, 1998).

In contrast to sulfate, which requires eight electrons for its complete reduction, ZVS requires only two electrons to be reduced to sulfide. Sulfur is produced in large quantities as a waste product and can be an attractive alternative to sulfate where the major or sole objective is to generate hydrogen sulfide. However, where the removal of sulfate from wastewaters is of primary concern, the use of ZVS is redundant. In addition, the insolubility of ZVS in water is a negative attribute, though acidophilic bacteria that use this solid phase material as either an electron donor or electron acceptor are able to facilitate direct contact with it and convert hydrophobic ZVS to a hydrophilic ('wetted') form (Dopson & Johnson, 2012).

The nature and amounts of suitable electron donors are also critical in determining the activities of acidophilic sulfate- and sulfur-reducing prokaryotes. While SRB as a group can use a variety of substrates as electron donors, this is generally much more restricted in terms of individual species. The most widely used organic electron donors are small molecular weight aliphatic acids and alcohols (Rabus *et al.*, 2013), though the former are problematic for acidophilic SRB, as discussed below. Bulky and complex organic substrates (e.g. straw and animal manures) are used in compost 'bioreactors' because of their (often) local availability and low cost (Neculita *et al.*, 2007). SRB are unable to degrade and utilize many complex (e.g. lignin and cellulose) biopolymers directly, and this is carried out by other microorganisms (eukaryotes and prokaryotes) also present in these constructed environments, generating organic materials that can be fermented (e.g. glucose produced by cellulose hydrolysis), and the fermentation products are thought to be the major electron donors used by the SRB in these systems. Apart from organic electron donors, hydrogen is widely used by SRB (Widdel & Hansen, 1992). This is a non-toxic, highly reduced and energetic substrate and is present in many autotrophic environments since it can be generated abiotically as well as biologically. Oxidation of hydrogen coupled to the reduction of sulfate generates only water and hydrogen sulfide as metabolic end products, and so no inhibition related to organic compounds would be anticipated. Hydrogen has been used to sustain full-scale sulfidogenic bioreactors (e.g. at the Nyrstar zinc refinery in the Netherlands; Meulepas *et al.*, 2009), though technical issues related to its flammability and mass transfer into aqueous phases are significant.

Organic acids, such as lactate, have widespread use as carbon and energy sources, and are used frequently to enrich, and to cultivate, SRB. However, acidophilic bacteria tend to be sensitive to organic acids, with concentrations of <1 mM often proving inhibitory or lethal. Many small molecular weight aliphatic acids exist predominantly as undissociated (and lipophilic) molecules in acidic waters such as AMD, and are able to pass freely through bacterial cell membranes. The internal pH of acidophiles tends to be circum-neutral which causes the organic acids to dissociate, and therefore a dis-equilibrium between internal and external concentrations of the (undissociated) acid, causing further influx. The resulting accumulation of protons within the bacterial cells can exceed the buffering capacity of cell cytoplasm and cause severe acidification. Gyure *et al.* (1990) found that 5 mM concentrations of organic acids inhibited sulfate reduction at pH 3.8, but concentrations of around 0.1 mM stimulated activity, while Fortin *et al.* (1996) noted that SRB isolated from minerals tailings were able use 1 mM acetate and formate as electron donors in medium adjusted to pH 7.5, but not that poised at pH 5.5 and below. In natural situations, concentrations of aliphatic acids in extremely acidic environments probably rarely exceed toxic levels, allowing them to be metabolized as with neutrophilic SRB. The situation is very different, however, in laboratories and constructed environments such as bioreactors, and different strategies need to be used to cultivate and sustain acidophilic SRB (Ñancucheo *et al.*, 2016).

Besides the nature and availabilities of electron donors and acceptors, other physio-chemical factors determine the activities of sulfidogenic SRB in natural and constructed environments. Temperature constraints impact the efficiencies of SRB bioreactors used for treating inorganic wastewaters. Daily and seasonal temperature fluctuations may affect the efficiency of biological sulfide production. In some areas, most notably in the high latitudes and at elevation, low temperatures ($<10^{\circ}\text{C}$) may prevail, which will limit *in situ* SRB activity. While psychrophilic SRB with temperature optima at around 20°C and which are active at $<10^{\circ}\text{C}$ have been isolated (Isaksen & Teske, 1996), all characterized acidophilic sulfidogenic bacteria are mesophilic or thermo-tolerant and acidophilic sulfidogenic archaea are mostly extreme thermophiles, as described below. Another factor that has been observed to affect the growth and activity of SRB is the availability of suitable surfaces for bacterial attachment and colonization (e.g. Bass *et al.*, 1996).

7.4.2 Acidophilic sulfate- and sulfur-reducing prokaryotes

Currently there are only four fully described and validated species of acidophilic SRB, though several others have been reported that appear to represent novel taxa. Sen and Johnson (1999) obtained the first documented pure culture acidophilic bacterial isolate (strain M1) from the White River draining the Soufriere Hills in Monserrat (WI) and a second, very closely related, isolate

(strain P1) from an abandoned copper mine in Wales. Sixteen years later, strain M1 was fully described and named as the type strain of *Desulfosporosinus acididurans* by Sánchez-Andrea *et al.* (2015), together with two related strains that had been isolated from the Rio Tinto (Spain). During this time, two other species of acidophilic SRB were described: *Thermodesulfobium narugense* which was isolated from a hot spring in Japan (Mori *et al.*, 2003), and *Desulfosporosinus acidiphilus* which was isolated from acid rock drainage in France (Alazard *et al.*, 2010). More recently, the novel species *Thermodesulfobium acidiphilum* (isolated from geothermally heated soil in Kamchatka, Russia) has been described (Frolov *et al.*, 2017). One of the characteristics of all acidophilic SRB described to date is the fact that they are moderate rather than extreme acidophiles (which have pH optima for growth at or below pH 3). The four species do, however, display different responses to temperature. *Td. narugense* is a moderate thermophile and grows between pH 4 and 6.5. *D. acidiphilus* grows between pH 3.6 and 5.5, and *D. acididurans* between pH 3.8 and 7. Both *Desulfosporosinus* (Gram-positive bacteria of the *Peptococcaceae* family and *Firmicutes* phylum, and characterized by endospore formation) species are mesophiles, and oxidize glycerol incompletely, generating acetic acid as a metabolic waste product. *Td. acidiphilum* grows at 37–65°C but has a similar pH range to the mesophilic isolates (3.7–6.5), though a closely related isolate (strain 3baa) has been reported to grow between pH 2.6 and 6.6 (Rüffel *et al.*, 2018).

Other *Peptococcaceae* strains of acidophilic SRB have been described, though not fully characterized. These include strains (Ñancucheo *et al.*, 2012) isolated from the Cantareras mine in Spain (Rowe *et al.*, 2007) and others isolated from the Rio Tinto (Sánchez-Andrea *et al.*, 2013). These are more closely related to the genus *Desulfatobacterium*, species of which reduce sulfite but not sulfate. However, the moderately acidophilic isolates can reduce sulfate and, in contrast to acidophilic *Desulfosporosinus* spp., oxidize glycerol completely to carbon dioxide. A novel genus ('*Desulfobacillus*') has been proposed for these isolates (Dopson & Johnson, 2012; Willis *et al.*, 2019).

While all moderately acidophilic SRB characterized to date are *Firmicutes*, there is circumstantial evidence to suggest that bacteria having this trait are much more diverse, phylogenetically. Molecular signatures (mostly 16S rRNA genes) of sulfate-reducing bacteria of the genera *Desulfatirhabdium*, *Desulfobulbus*, *Desulfobacterium*, *Desulfobacca*, *Desulfomonile* and *Desulfatirhabdium* have been detected in a variety of low pH sulfidogenic bioreactors (Montoya *et al.*, 2013; Sánchez-Andrea *et al.*, 2014). However, until these bacteria are isolated and the tolerance to pH tested in axenic cultures in the laboratory, it remains unclear whether they are genuine acidophiles or (like many SRB in environmental situations) occupy and sustain higher pH micro-niches within these bioreactors.

Many of the moderately acidophilic SRB that have been described can also catalyze the dissimilatory reduction of ZVS to hydrogen sulfide. Some species of

acidophiles can also grow under anaerobic conditions via sulfur respiration, but do not reduce sulfate. Interestingly, most of these (exclusively archaea) were characterized long before the first acidophilic SRB, and are extreme acidophiles. Both *Thermoplasma acidophilum* (Darland *et al.*, 1970) and *Thermoplasma volcanium* (Segeer *et al.*, 1988) are moderately thermophilic facultative anaerobic euryarchaeotes that couple the oxidation of organic carbon to the reduction of either oxygen or elemental sulfur. Other ZVS-reducing acidophilic archaea are crenarchaeotes, and include *Acidianus brierleyi* and *Acidianus infernus*, both of which can oxidize as well as reduce ZVS, depending on oxygen availability (Segeer *et al.*, 1986), *Sulfurisphaera ohwakuensis* (Kurosawa *et al.*, 1998) and the obligately anaerobic thermo-acidophile *Stygiolobus azoricus* (Segeer *et al.*, 1991). The only currently classified species of ZVS (non-sulfate)-reducing acidophilic bacteria is *Desulfurella amilsii* (Florentino *et al.*, 2016). This is a thermo-tolerant (growth between 20° and 52°C), acid-tolerant (pH range of 3 to 7, with growth optimum between 6 and 6.5) bacterium that can also reduce thiosulfate and mediate the disproportionation of ZVS. Another ZVS-reducing acidophilic bacterium described though not yet classified is strain I2511 (Holanda & Johnson, 2020). This isolate is a mesophilic *Firmicute*, distantly related to *Alicyclobacillus* spp. that can grow by ZVS- or iron-respiration at an optimum and minimum pH of ~3.5 and ~2.5, respectively.

7.5 BIOENGINEERING APPROACHES FOR REMEDIATING SULFATE-RICH MINE WATERS

7.5.1 Constructed wetlands

Most biological processes used for remediation of mine water came about as result of observations of these processes in the natural environment. Constructed wetlands, for example, mimic their natural counterparts by creating an engineered ecosystem, which provides required redox, acid neutralization and precipitate settling functions (Skousen *et al.*, 2017). In the late 1970s, Huntsman *et al.* (1978) reported that outflows from naturally dominated *Sphagnum* bogs displayed improved water chemistry compared with inflowing AMD from a coal mine in Ohio, USA. The increased pH of the outflow was accompanied by decreased concentrations of sulfate, iron and other metals. Wetlands are highly complex ecosystems, and modifications of water chemistry may occur due to several mechanisms, including dilution, precipitation (by oxidative and reductive mechanisms), adsorption, and uptake by biomass.

There are three main types of constructed wetland, which operate on radically opposed biogeochemical activities based on either oxidative (aerobic wetlands) or reductive (anaerobic and vertical flow wetlands/reducing and alkalinity producing systems (RAPS)) processes. The main mechanisms involved in these systems include: (i) biological metal oxidation, precipitation and capture,

(ii) biological sulfate reduction and precipitation of metal sulfides in an organic matter layer, (iii) complexation with organic matter, (iv) sorption, and (v) direct uptake by plant roots (Skousen *et al.*, 2017). The main objective of aerobic wetlands is to promote iron oxidation, hydrolysis and metal hydroxide precipitation. Aerobic wetlands are relatively shallow systems, within which acid- and metal-tolerant macrophytes are planted in soil or other substrate, to control the water flow and to filter and stabilize iron precipitates. These systems remove metals by slowing the water flow and allowing for oxidation and may be used in tandem at a treatment site, often in association with anoxic limestone drains. *Sphagnum* mosses have proved to be somewhat sensitive to changes in AMD chemistry and accumulation of iron within constructed wetlands, and therefore cattails (*Typha latifolia* and *Typha orientalis*) are generally the preferred vegetation. The oxidation and precipitation of iron can result in significant production of proton acidity as AMD flows through aerobic wetlands, which slows down the rate of spontaneous (chemical) oxidation.

‘Compost bioreactors’ (anaerobic wetlands) function by actively generating alkalinity as a result of microbiological reductive activities, and are therefore commonly used to treat net-acidic waters. Since organic electron donors fuel these processes, various organic materials, such as cow and horse manure, spent mushroom compost, peat, sawdust and wood chips, have been used as substrates in compost wetlands. The choice of organic materials used varies according to their effectiveness and local availability. In some cases, anaerobic wetland substrates are mixed with limestone, which contributes to continuous alkalinity supply since there is neither Fe^{2+} oxidation nor Fe^{3+} precipitation to coat the limestone surface. The main mechanisms involved in anaerobic wetland systems are the formation and precipitation of metal sulfides, removal of sulfate and alkalinity generation by sulfate reduction reactions and continuous generation of carbonate alkalinity. Dissolution of basic minerals within the compost mix will also contribute to acid neutralization. The reduction of both iron and sulfate are important processes in anaerobic wetlands. In some systems, cattails and other emergent vegetation provide a continuous supply of carbon to the system. However, root penetration may introduce oxygen into the anaerobic zone and reduce the effectiveness of the reductive processes. In some systems, surface plants are excluded, and the anaerobic ‘cell’ is so constructed as to limit any oxygen diffusion. Microbial populations that develop in anaerobic wetlands are, at least in the initial phases, the indigenous populations of the composting materials, and direct inoculation tends not to be used. Microorganisms, including obligate and facultative acidophilic and acid-tolerant bacteria present in inflowing AMD, may also establish in these constructed environments.

Vertical flow wetlands/RAPS (Figure 7.4) were developed in the late 1980s (Younger *et al.*, 2002). These vertical ponds have perforated pipes embedded in a limestone layer at the bottom of the system and a layer of organic matter substrate at the top. The AMD flows downwards through the organic substrate



Figure 7.4 A reducing and alkalinity producing (RAP) wetland constructed to treat acidic, iron-rich water draining an abandoned coal mine (Tan-y-Garn, South Wales).

layer, where O_2 is consumed, generating anoxic conditions. Sulfate reduction contributes to acid neutralization, and ferric iron reduction eliminates potential coating of the underlying limestone, which provides further acid neutralization. Aerobic wetlands or settling ponds may be used to precipitate iron and manganese downstream of compost-based wetlands.

7.5.2 Bioreactor systems

While it is generally accepted that biological processes (mostly driven by microorganisms) are the most significant involved in remediating AMD in natural and constructed wetlands, the fact that these ecosystems do not lend themselves to ready control and manipulation means that their performance is difficult to predict and is subject to seasonal and other variations (Johnson & Hallberg, 2002). Biological treatment of AMD and similar wastewaters has several major advantages over active (chemical) treatment. Operating costs and footprint of the latter are high, metal removal efficiencies tend to be relatively low, bulky sludges requiring dewatering and adequate disposal are produced, and all potentially valuable metals which accumulate in the sludge are eventually lost or remobilized. In contrast, bioreactor systems facilitate the recovery of chalcophilic metals (and of sulfur, when integrated with elemental sulfur production), the sludge produced is far denser (some 6–10 fold), less voluminous and more stable (Neculita *et al.*, 2007), and sulfate concentrations can be lowered to potable water levels. On the other hand, they may require fairly sophisticated and expensive engineering systems, though this can be partially offset by the commercial value

of the metals recovered. In the past few decades, various biotechnologies have been developed based on isolating 'useful' microorganisms from the environment and engineering a controlled system to promote their growth and activities. Generally, these involve the use of bioreactors in order to neutralize AMD, precipitate metals as oxy-hydroxide phases (mostly iron) or as sulfides (such as copper and zinc), recover potentially valuable metals and reduce the amount of waste that requires disposal since water and metals may be recycled and reused (Johnson & Sánchez-Andrea, 2019).

Biological sulfate reduction has been widely applied for remediating metal-, metalloid- and sulfate-rich mine waters using laboratory-scale systems. This technology harnesses the metabolic diversity and versatility of sulfate- and sulfur-reducing bacteria in order to design bioremediation processes. Biogenic or chemical sulfide not only remove potentially toxic metals from wastewaters but can also provide a selective metal precipitation (Ñancucheo *et al.*, 2012; Santos and Johnson, 2018). The metal removal is based on the reaction of hydrogen sulfide with some metal cations (usually metals with +2 oxidation states) to form insoluble metal sulfide precipitates, such as Zn, Co, Cu, Ni, and Cd (Lewis, 2010). There are two commercial-scale processes based on microbial mediated sulfate reduction using bioreactor systems for the treatment of acidic wastewaters from metal mines and related situations: the *BioSulphide*[®], developed by BioteQ Environmental Technologies Inc., Canada and the *SULFATEQ*[™] by Paques, the Netherlands. In both, the central process is the generation of sulfide by neutrophilic SRB with the main objective being to lower the levels of toxic metals to environmentally acceptable concentrations. In addition, these bioreactor systems offer the opportunity to lower sulfate concentrations, precipitate metals selectively and minimize/eliminate the production of waste sludges.

The *BioSulphide*[®] process essentially utilizes biogenic H₂S generated by the reduction of ZVS by sulfur-reducing bacteria (biological stage) to remove transition metals from acid mine drainage (chemical stage). In this case, biogenic H₂S is delivered to a contactor stirred tank containing AMD (Figure 7.5). By carefully controlling pH and sulfide concentrations, the selective precipitation of a particular metal can be achieved due to differences in the solubility product of metal sulfides, e.g. copper in a waste stream containing a mixture of iron, zinc and copper (Ashe *et al.*, 2008). The sulfide concentrate is recovered in a clarifier and subsequently dewatered using a filter press. The waste stream can then proceed to a second precipitation tank where further metal precipitation (e.g. zinc) can be induced by controlling pH and flow rates. In cases where more than one metal product is to be recovered, multiple contactors and clarifiers can be used.

In 2001, the first commercial *BioSulphide*[®] process plant was established at the Caribou Mine in New Brunswick (Canada) designed to remove Cu, Zn, Cd and Pb from AMD upstream of the existing lime plant. Due to the success of the plant in demonstrating its technical feasibility, other full-scale commercial plants were commissioned, for example, in North America at the Copper Queen Mine in

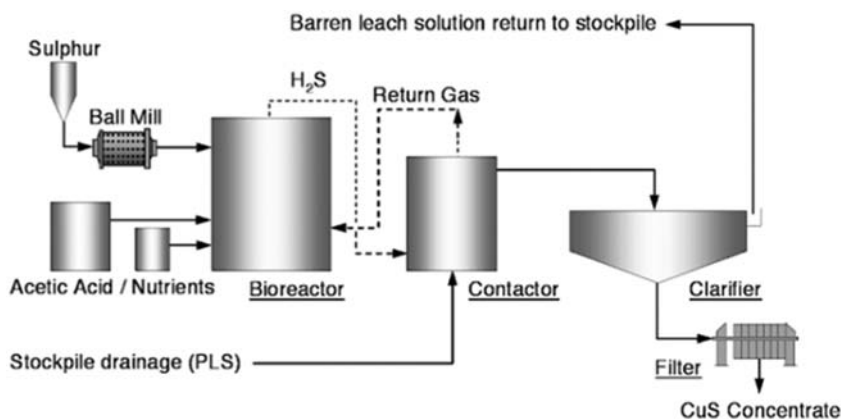


Figure 7.5 Simplified flowsheet of the *BioSulphide*[®] process at the Copper Queen Mine. (Ashe *et al.*, 2008, with permission). PLS, pregnant leach solution.

Bisbee, Arizona and at the Raglan Nickel mine in northern Quebec (Canada). The Bisbee Project started in 2004 producing a high-grade copper concentrate, typically containing >40% copper. About 974,000 m³ wastewater were treated using this technology (with >99% copper recovery) and 492,000 pounds of copper were produced in the year of 2012. The operations at the Bisbee plant were suspended in 2013 and the plant was decommissioned in 2015. The Raglan Project, also established in 2004, has been operated as a seasonal water treatment plant at an active nickel mine in the Canadian Arctic region. The nickel sulfide produced in the process is up to 40% (dry weight) and has been successfully incorporated with the conventional flotation concentrate produced at the Raglan mine (Bratty *et al.*, 2006). The operation contract of this water treatment plant has been extended until 2020.

The *SULFATEQ*[™] process utilizes two different biological processes, in contrast to the single bioprocess in the *BioSulphide*[®] technology. As before, sulfide is generated in a sulfate-reducing bioreactor in the first biological stage of the operation (again generating net alkalinity and promoting the precipitation of chalcophilic metals in the wastewater). The H₂S generated is directed to a contactor where zinc is precipitated as sulfide, which is collected and reused in smelters. Using this technology, 95% of the dry sludge generated is zinc sulfide. The second biological stage involves the oxidation of the excess of hydrogen sulfide, present as HS⁻, to ZVS under oxygen-controlled conditions by sulfide-oxidizing bacteria in a S⁰-generating bioreactor (Boonstra *et al.*, 1999). Two useful products are therefore produced: metal sulfides, which may be processed to recover the metal(s) concerned, and hydrophilic sulfur, which may be used to produce sulfuric acid or as an agrochemical by-product. This technology has been demonstrated at a number of pilot-scale operations and,

since 1992, the first full-scale plant has been in operation to remediate acidic metal- and sulfate-rich groundwater at the Nyrstar zinc refinery in Budel-Dorplein (The Netherlands). Over 100 years of zinc refining at the site had produced severe acid and metal contamination of groundwater and soils adjacent to the site, and there was concern about the pollution plume spreading to nearby aquifers, which were sourced for drinking water. A hydrogeological containment system was designed, in which the contaminated groundwater is pumped to the surface, processed so that its quality falls within the limits set by the Dutch authorities, and then discharged into a nearby river. Various options were considered, and the decision was made, following extensive laboratory- and pilot-scale testing, to install a biological treatment facility. The system installed utilized an upflow anaerobic sludge blanket (UASB) reactor for the sulfate reduction step, and a submerged fixed film reactor for the aerobic conversion of sulfide to elemental sulfur. In the processed groundwater, typical effluent concentrations of zinc, cadmium and sulfate are, respectively, <0.05 , <0.001 and <200 mg/L. Annually, around 300 tons of zinc are recovered using the *SULFATEQ*TM biotechnology at the Nyrstar zinc refinery.

Both of the above processes use bacteria that are sensitive to even moderate acidity and relatively small concentrations of some transition metals, and therefore these bacteria need to be protected from direct exposure to AMD. As a result, the engineering complexity of the reactor systems can cause financial implications for both their capital and operating expenditures. The adaptation of neutrophilic species of SRB to moderately acidic conditions has been reported as an alternative approach, though studies have shown that incrementally lowering the bioreactor pH may negatively impact both bacterial activity and biomass yield (Bijmans *et al.*, 2008). More recently, the development of novel biological sulfate reduction systems using species of acidophilic/acid-tolerant sulfate-reducing bacteria have been described (Ñancucheo & Johnson 2012, 2014; Santos & Johnson, 2017). In an acidophilic sulfate-reducing bioreactor contact between AMD and the bacterial consortium is possible, and therefore a single bioreactor can be used simultaneously to grow biomass and to precipitate target metals. Since the reaction catalyzed by these bacteria consumes protons, the bioreactors can be operated as continuous flow systems where pH is maintained by balancing the consumption of protons by the inflow of AMD. The system operates with minimal control and input of reagents and is robust throughout continuous testing using laboratory modules and different water chemistries (Ñancucheo *et al.*, 2012; Ñancucheo & Johnson, 2014; Santos & Johnson, 2018), and can therefore be considered as an attractive alternative to existing technologies.

Laboratory-scale systems have shown that biosulfidogenesis is feasible either as a stand-alone technology or as part of integrated systems for AMD treatment and the removal of metals can occur both in-line (precipitation within the reactor vessel) and off-line. However, the design and configuration of the system will depend mainly on the chemistry of the wastewater being treated. Ñancucheo and Johnson (2014) used

a sulfidogenic bioreactor, maintained at low pH, to lower concentrations of sulfate from two extremely low pH mine waters that contained small concentrations of iron, but no other chalcophilic metals. Over 98% of sulfate was removed from one and 50–60% from the other. The hydrogen sulfide generated in this process was converted to colloidal sulfur in an off-line vessel. Santos and Johnson (2018) described the application of a single sulfidogenic bioreactor housing a consortium of acidophilic SRB and other acidophiles, operated at low pH (4–5) used to remove transition metals (copper, nickel, cobalt, and zinc) from a moderately acidic (pH 5) drainage stream at an operating copper mine in northern Brazil. Copper was the most abundant transition metal in the mine water (7.5 mM) while concentrations of the other transition metals were <0.25 mM. The treatment involved two stages: (i) off-line copper recovery via a stream of H₂S-containing gas generated by the bioreactor and (ii) in-line removal of nickel, zinc and cobalt from the partially processed water, which was amended with glycerol, yeast extract and basal salts and fed directly into the bioreactor. All four metals were successfully removed with recovery of a high-grade CuS (Figure 7.6).

Hedrich and Johnson (2014) proposed an integrated system designed to combine remediation of and metal recovery from AMD at the Mauriliden mine in Sweden. This mine water contained ~7 mM zinc and iron and smaller concentrations of arsenic, cadmium, aluminum and manganese. This integrated system comprised of four main steps. First, soluble arsenic was removed from the mine water upstream of the other modular bioreactors by adsorbing the negatively charged arsenate onto the positively charged schwertmannite in order to avoid the co-precipitation of this toxic metalloid in the following modules and contamination of the metal sulfides recovered. The second step involved a bioreactor used to oxidize ferrous iron in the AMD to ferric, and to produce schwertmannite, a small portion of which (~11%) was used to remove As in the previous step. Soluble copper and cadmium were removed in the next stage by reacting with biogenic H₂S in an off-line reactor vessel. Lastly, the Cu/Cd-free mine water was then amended with glycerol and fed into an acidophilic sulfidogenic bioreactor. Most of the zinc was removed by maintaining the pH of the unit at 4.0, which also avoided co-precipitation of aluminum and manganese.

Elsewhere, Falagán *et al.* (2017) described the use of a low pH sulfidogenic modular system to remove soluble aluminum from synthetic acidic Al-rich mine waters. The precipitation of aluminum hydroxysulfate minerals (hydrobasaluminite and felsőbányaite) from soluble aluminum was mediated by sulfate-reducing bacteria, which utilized the protons released during the formation of these aluminum hydroxysulfates as well as those present in the feed liquor in the sulfate reduction process (equation (7.8)), thereby facilitating the continuous hydrolysis and precipitation of aluminum. The bioreactor was dominated by *D. acididurans* and two species of facultative anaerobic non-sulfidogens *Acidocella aromatica* and *Acidithiobacillus ferrooxidans*. Recently, a modified version of the acidophilic sulfidogenic bioreactors was designed to combine the

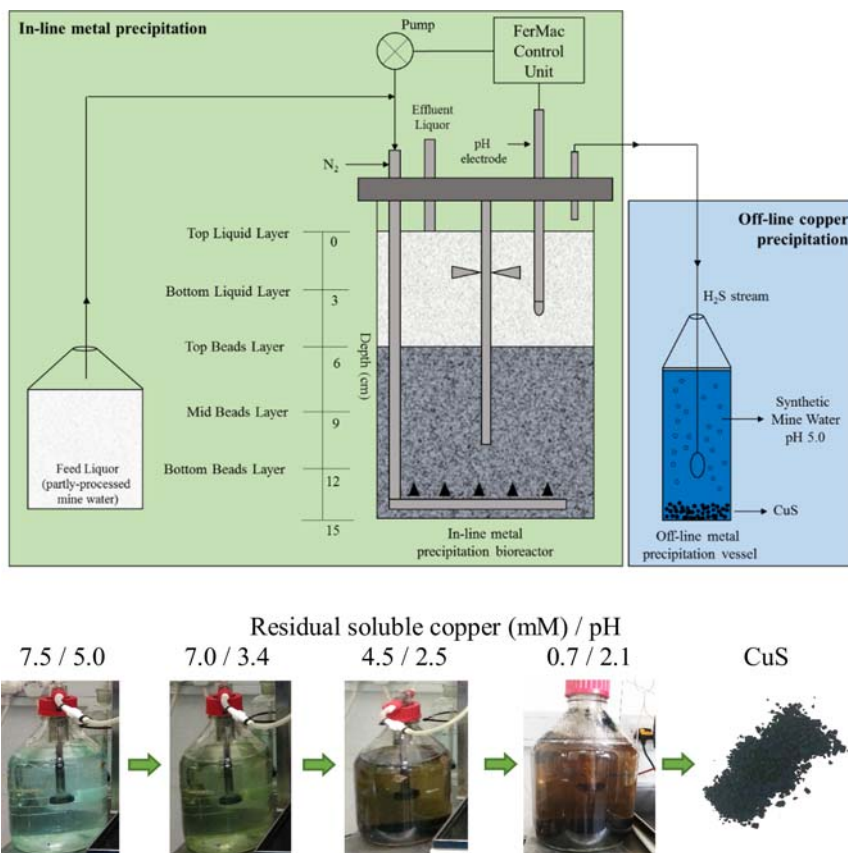


Figure 7.6 Top: Schematic representation of the low pH sulfidogenic bioreactor. Excess hydrogen sulfide produced in the bioreactor was delivered to an off-line vessel, containing synthetic mine water, in order to mediate selective removal of copper. Following copper precipitation, the more acidic partly-processed water was used as feed liquor for the bioreactor. Bottom: Time-lapse images of different stages of copper mineralization in the off-line vessel. X-ray diffraction analysis of the solid phase product confirmed that it was covellite (CuS; Santos & Johnson, 2018).

use of sulfate and ZVS as electron acceptors. The ‘hybrid’ sulfidogenic bioreactor (HSB) was populated with acidophilic sulfidogens (*D. acididurans* and *Peptococcaceae* CEB3, which can reduce sulfate as well as ZVS), other acidophilic bacteria (*Actinobacterium* AR3, *At. ferrooxidans* and *Ac. aromatica*) and also a novel *Firmicute* that does not reduce sulfate, but reduces ZVS. The HSB effectively removed zinc from circum-neutral pH mine-impacted waters from abandoned Zn/Pb mines in the UK (Holanda & Johnson, 2020).

7.5.3 Pros and cons of the options available for remediating acidic sulfurous wastewaters

Of the three approaches currently used to remediate ‘inorganic’ wastewaters such as AMD, two make use of SRB to generate alkalinity, remove heavy metals and lower sulfate concentrations. All three approaches have their limitations and detractions. Abiotic (lime) treatment is expensive to maintain, produces a bulky sludge and generally does not lower sulfate concentrations very effectively. Wetlands can be constructed only where there is sufficient suitable land area available. Their performance tends to be variable, and net sulfate reduction may not always be apparent, particularly in older systems. Advanced technology systems using SRB bioreactors have several advantages, including consistency of performance and the potential for recycling materials (metals and sulfur) from the wastewaters which have commercial value. Bioreactor plants, however, require significant start-up capital and current systems use pure-grade chemicals (small molecular weight organic materials such as ethanol or methanol, or hydrogen) as electron donors for the bacteria. Bioreactor systems do, however, offer the major advantages of control, and potential to significantly lower sulfate concentrations and to selectively precipitate metals. By being unique among the available options in generating end products that can be re-used and recycled (e.g. ZVS and transition metal sulfides), this biotechnology would seemingly be far better suited to a future where resource recovery combined with environmental protection is critical.

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